

**CHAPTER 12**

# **Histopathological Examination of a ‘Simultaneously folded’ Method with Paste and Combined Root Canal Filling Methods in Dog Teeth**

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## **《Abstract》**

To improve the method of root canal filling, the authors performed a histopathological study using a novel system named the ‘simultaneously folded’ root canal filling method. This system calls for applying Vitapex paste near the area of the apical foramen followed by filling the remainder of the root canal by means of either the combination of gutta-percha point and KEZ endodontic cement (dry components: 40% zinc oxide, 20% calcium hydroxide, 20% iodoform and 20% rosin; liquid components: 90.5% eugenol 90.5%, 9.0% rosin and 0.5% setting agent) or Neodyne zinc-oxide eugenol cement. The authors compared the healing effects of this method with those resulting from a combined root canal filling method employing gutta-percha point and Neodyne endodontic cement. The root canals of 119 dog teeth were perforated by means of rotary cutting instruments. Pulp chambers were exposed and allowed to remain open for thirty days. Engine reamers were used to perforate the apical portions. They were cleaned with sodium hypochlorite and saline solutions. Fifty-one of the canals were filled using our ‘simultaneously folded’ technique with Vitapex, gutta-percha point and KEZ endodontic cement. Forty-two canals were filled using the ‘simultaneously folded’ technique with Vitapex, gutta-percha point and Neodyne. Twenty-six canals were filled using a combined root canal filling method with gutta-percha point and Neodyne. Three months later, the dogs were sacrificed and specimens were prepared for histopathological observation. Results showed that the ‘simultaneously folded’ root canal filling method resulted in a peculiar healing process featuring a cement barrier without ankylosis.

Apparently, this method accelerated the closure of the apical foramen and regeneration of alveolar bone. Consequently, the 'simultaneous folded' root canal filling method is recommended for clinical application.

## Introduction

Root canal treatment is essential for the management of infected root canal after pulp extirpation, as was advocated by Rickert and Dixon (1931) [1] and subsequently pursued by many other researchers [2-24]. We have also done relevant studies in this field [3] [4]. Actually, the origin of root canal filling was said to begin in the year 1757, when Bourdet utilized gold as root canal filling material [5]. Since then, systems have utilized a number of root canal fillings, which are roughly divided into four groups: paste, solid, combination of different pastes, and combination of paste and solid. Those systems were justified over many years of clinical experiences and research, but new drugs and materials are now on their way, which have made improvements in the surgical field. Thus, it is difficult to conclude by clinical conditions the outcome of any root canal filling method or surgical procedure.

In this chapter, we have attempted to investigate a new root canal filling method, specifically a new root canal filling method for the treatment of infected root canals using histopathological examination. We already conducted a series of studies regarding the conditions of infected root canals [3, 4, 6] and determined the incidence of certain conditions. In the study of root canal fillings, we used paste in infected root canals after surgical operation and the cleaning of the canal. The study employed the 'simultaneously folded' root canal filling method [7] (Refer to Figure 24). This method is in the fourth category, as previously stated; it is new and different in several ways, utilizing the best of the traditional method with the purpose of saving the tooth by root canal treatment. The use of paste as a root canal filler, like calcium hydroxide, established a significant modification of the root apex inducing closure. However, adopting this method has some disadvantages [8-17]. Finally, from the four systems we selected the paste system, which specifically confirmed sealing of the canal after a certain period, then switched to combined solid and paste. This 'simultaneously folded' root canal filling requires a period of several months to fill the root canal, and to shorten the treatment period, the operation can be performed all at once, making it useful in clinical practice. We have already completed experiments using 'simultaneously folded' root canal filling, and at the same time confirmed the efficacy of Vitapex applied to the root tip [4, 18, 19]. Closure of the root canal space has been confirmed by this root canal filling method and KEZ [3] and with Neodyne [20]. Silicone root canal fillers and Vitapex were not used in 'simultaneously folded' root canal filling because the paste form of root canal filler can dramatically increase and its operation is performed easily. Application of the paste can be controlled to some extent, which gives good results [4, 44-46]. Combined root canal filling with gutta-percha points and KEZ endodontic cement empirically used for infected root canals has been reported to demonstrate superior performance [3]. Moreover, the approximate powder components of KEZ endodontic cement and Vitapex cause no problems of any kind of root canal filling material even if the mix is stacked because Neodyne has been incorporated. However, the combination of materials has not been studied in 'simultaneously folded' root canal filling as well as the healing progress using this root canal filling method compared to conventional method.

## Materials and methods

### Experimental animals

The animals used in this study were 11 male and female healthy dogs (hybrid), more than 1 year of age, weighing 5.5 to 24kg. A total of 66 mandibular molar teeth with 119 root canals were obtained.

Samples were divided into 3 groups. The first group was filled with Vitapex (Neo Chemical Industries Co.), gutta-percha points and KEZ endodontic cement using the 'simultaneously folded' technique (VKP group). The second group was filled with Vitapex, gutta-percha points and Neodyne (Pharmaceutical Industry Co.) using the 'simultaneously folded' technique (VNP group). The third group was filled with Neodyne and gutta-percha points using a conventional combined root canal filling technique (NP group). The composition of KEZ endodontic cement, Vitapex and Neodyne are shown in Tables 1, 2, 3, respectively.

**Table 1** Components of Endodontic Cement KEZ

[Powder]	%
Zinc Oxide	40.0
Iodoform	20.0
Calcium Hydroxide	20.0
Rosin	20.0
[Liquid]	
Eugenol	90.5
Rosin	9.0
Hardening	0.5

**Table 2** Components of Root Canal Filling Paste Vitapex

	%
Calcium Hydroxide	30.3
Iodoform	40.4
Silicone Oil	22.4
Others	6.9

**Table 3** Components of Root Canal Filling Material Neodyne

[Powder]	%
Zinc Oxide	60.0
Rosin	25.0
Calcium Hydroxide	15.0
[Liquid]	
Eugenol	100.0

### Experimental procedures

Prior to the experiment, the dogs were first subjected to femoral intravenous anesthesia with sodium pentobarbital (Nembutal, Dainippon Pharmaceutical Co., Ltd.) according to weight. The subjects were fixed on an operating table for radiograph taking. X-ray films were fabricated using a fixed tray for uniformity so as to measure the relation of the location of root tip and lesion before and after surgery. After root canal therapy, the formation of periapical lesion was confirmed by the change in radiographic contrast. Radiographic observation of the healing process was confirmed in order to establish a relationship with histopathological findings. From the time of formation of lesion at the root tip until after root canal filling 3 months later, radiographs were taken at least 4 times.

After pre-operative radiograph, the operating field was disinfected with iodine and 70% ethanol. Then after abrasive point (Matsukaze Manufacturing Co.) or diamond cutting bur (Matsukaze Manufacturing Co.) was used to remove the cusp, a no 2 or 3 round bur was used to create access until the pulp chamber was completely opened. The pulp cavity was left opened for a month. Radiograph was performed under general anesthesia using sodium pentobarbital a month after to confirm the formation of apical lesion. After sterilization with iodine and 70% ethanol, a hole was created at the root tip using engine reamer no 15 to 30 (Zipperer Inc.). The canal was irrigated with 1% sodium hypochlorite and physiologic saline solution and dried with paper points (Zipperer Inc.). For the VPK group, 28 teeth with 51 root canals were treated with Vitapex applied at the apex and the canal was filled using multiple gutta-percha points and KEZ endodontic sealer, and it was sealed with zinc phosphate lining cement and amalgam restoration. For the VNP group, 22 teeth with 42 root canals were treated with Vitapex applied at the apex followed by multiple applications of gutta-percha and Neodyne endodontic cement, and sealed with zinc phosphate lining cement and amalgam restoration. In contrast to the NP group, 16 teeth with 26 root canals were treated with combined gutta-percha points and Neodyne, sealed with zinc phosphate cement, and given amalgam restoration. Immediately after obturation, the condition of the root canal treatment was confirmed by radiograph. The subjects were subjected to 3 months observation.

### Pathological findings

Comprehensive histological observations were made on the periapical tissues and root apex; various changes were noted and the results were determined according to a classification consisting of 6 categories with 5-point scores as previously reported (Teramoto [3], Shibuya [4]). The traditional rating of good, fair and poor was clearly related to the 6 categories.

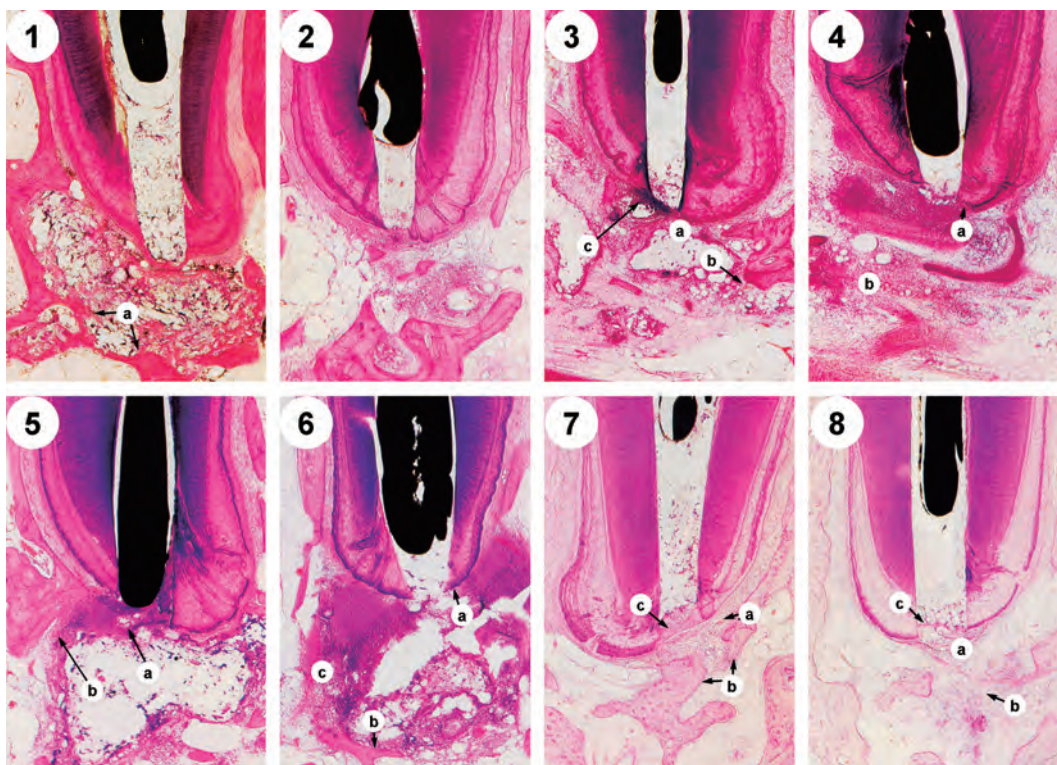
#### Pathological findings of VPK group

Table 4 shows the pathological results of this group. Typical examples of this experimental group are described below.

Figure 1 shows the histological view of the mesial root of right mandibular 3<sup>rd</sup> premolar 83 days post-operatively. The previously resorbed alveolar bone was replaced with marked proliferation of bone-like or irregular calcified tissues. Histological findings showed good results, and a score of 5 points was given.

Figure 2 shows the histological view of the distal root of right mandibular 3<sup>rd</sup> premolar 83 days post-operatively. The apex has been restored to nearly normal state with active bone regeneration. Also, the periodontal ligament around the root tip is almost normal. The hole created at the root tip was almost closed by cementum. A score of 4 points was given.

Figure 3 shows the histological view of the mesial root of right mandibular 3<sup>rd</sup> premolar 84



- Figure 1** Experimental VKP group, Case 3, Period 83 days, Histopathologically excellent, mandibular right 3<sup>rd</sup> premolar mesial root (a: alveolar bone regenerative deposition).
- Figure 2** Experimental VKP group, Case 83, Period 83 days, Histopathologically excellent, mandibular right 3<sup>rd</sup> premolar distal root.
- Figure 3** Experimental VKP group, Case 34, Period 84 days, Histopathologically good, mandibular right 3<sup>rd</sup> premolar mesial root (a: apical hard tissue resorption; b: hemorrhage; c: superlative inflammation).
- Figure 4** Experimental VKP group, Case 40, Period 83 days, Histopathologically good, mandibular right 3<sup>rd</sup> premolar mesial root (a: apical hard tissue resorption; b: scar tissue).
- Figure 5** Experimental VKP group, Case 35, Period 84 days, Histopathologically poor mandibular right 3<sup>rd</sup> premolar distal root (a: superlative inflammation; b: hyperemia).
- Figure 6** Experimental VKP group, Case 49, Period 92 days, Histopathologically poor, mandibular right 3<sup>rd</sup> premolar distal root (a: apical hard tissue resorption; b: alveolar bone resorption; c: inflammation cell infiltration).
- Figure 7** Experimental VNP group, Case 29, Period 92 days, Histopathologically excellent, mandibular left 3<sup>rd</sup> premolar mesial root (a: hyperemia; b: alveolar bone regeneration/deposition; c: complete cementum bridge formation).
- Figure 8** Experimental VNP group, Case 28, Period 92 days, Histopathologically excellent, mandibular left 2<sup>nd</sup> premolar distal root (a: inflammatory cell infiltration; b: scar tissue; c: un-complete cementum bridge formation).

days post-operatively. The hole near the root tip still remained but a small portion was closed by cementum. Bleeding and scarring around the root tip was also seen. A score of 3 was given.

Figure 4 shows the histological view of the mesial root of right mandibular 3<sup>rd</sup> premolar 83 days post-operatively. Scarring at the root end was observed to some extent with bone regeneration. A tendency towards bone regeneration was also noted and a score of 2 points was given.



Figure 5 shows the histological view of the distal root of right mandibular 3<sup>rd</sup> premolar 84 days post-operatively. Traces of remaining inflammatory cell infiltration at the periphery of the extravasated paste could still be found. On the other hand, the hole below the root tip was still present, and resorption of the root tip and hard tissue around it could be observed. Ongoing hard tissue regeneration at the mesial root tip was also observed. This case was given a score of 1 point.

Figure 6 shows the histological view of the distal root of the right mandibular 3<sup>rd</sup> premolar 92 days post-operatively. Periapical lesion surrounded by strong pus formation was seen at the traces of the paste that extravasated. Alveolar bone resorption could be seen in most parts, though there was a slight tendency to scarring around the lesion. This case had poor results and a score of 0 points was given.

### **Pathological findings of VNP group**

The following show typical examples of VNP experimental group. Figure 7 shows the histological view of the mesial root of the left mandibular 3<sup>rd</sup> premolar 92 days post-operatively. There was no further inflammatory change in the periodontal tissue at the root tip. Marked alveolar bone regeneration with scarring was even observed. The root tip, as well as the resorbed parts, was completely closed by cementum. Because the case was fully restored, a score of 5 points was given.

Figure 8 shows the histological view of the distal root of left mandibular 2<sup>nd</sup> premolar 92 days post-operatively. A lesion at the periodontal tip was clearly evident. It consisted of blood vessels in the connective tissue; an active form of healing process from the inner layer to the periphery was noticed with alveolar bone regeneration towards the center. A score of 4 points was given.

Figure 9 shows the histological view of the distal root of the left mandibular 4<sup>th</sup> premolar 92 days post-operatively. Periodontal tissues at the root tip were in the healing process. However, just below the hole, a considerable degree of inflammatory cell infiltration remained and so a score of 3 points was given.

Figure 10 shows the histological view of the distal root of the right mandibular 3<sup>rd</sup> premolar 2 days post-operatively. Marked tissue changes and damage still remained and so a score of 2 points was given.

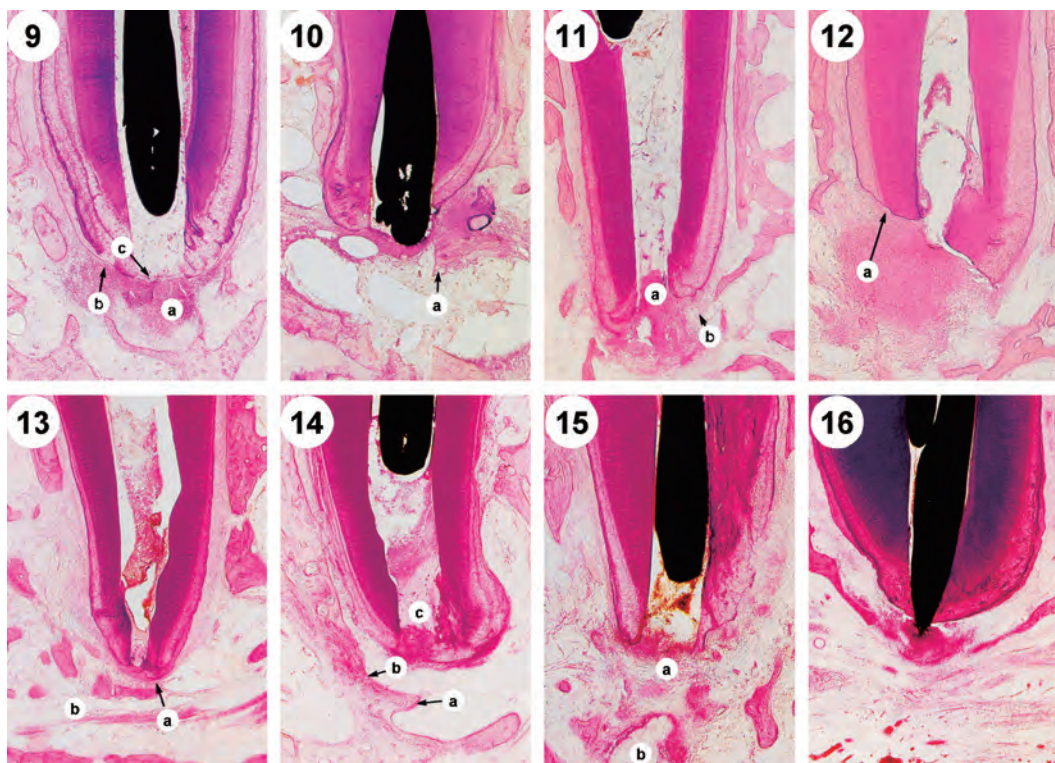
Figure 11 shows the histological view of the mesial root of the left mandibular 3<sup>rd</sup> premolar 83 days post-operatively. Hard tissue resorption markedly occurred at the root tip hole with proliferation of granulation tissue with alveolar bone resorption and so a score of 1 point was given.

Figure 12 shows the histological view of the mesial root of the left mandibular 2<sup>nd</sup> premolar 83 days post-operatively. Severe root tip resorption, considerable suppuration in some parts of periodontal tissues and inflammatory cell infiltration were all encountered. A score of 0 points was given.

### **Pathological findings of NP group**

The following are typical examples of this group. Figure 13 shows the histological view of the mesial root of the right mandibular 3<sup>rd</sup> premolar 83 days post-operatively. The periodontal root tip is in a state of recovery. In addition, the hole was completely closed by cementum formation and so a score of 5 points was given.

Figure 14 shows the histological view of the mesial root of the right mandibular 2<sup>nd</sup> premolar 92 days post-operatively. The periodontal root tip nearly recovered to physiologic



**Figure 9** Experimental VNP group, Case 32, Period 92 days, Histopathologically good, mandibular left 4<sup>th</sup> premolar distal root (a: inflammation cell infiltration; b: apical hard tissue regeneration/deposition; c: cementum bridge formation).

**Figure 10** Experimental VNP group, Case 38, Period 92 days, Histopathologically poor, mandibular right 3<sup>rd</sup> premolar distal root (a: alveolar bone regeneration/deposition).

**Figure 11** Experimental VNP group, Case 3, Period 83 days, Histopathologically poor, mandibular left 3<sup>rd</sup> premolar mesial root (a: root canal polyp formation; b: scar tissue).

**Figure 12** Experimental VNP group, Case 1, Period 83 days, Histopathologically poor, mandibular left 2<sup>nd</sup> premolar mesial root (a: apical hard tissue resorption).

**Figure 13** Control NP group, Case 2, Period 83 days, Histopathologically excellent, mandibular right 3<sup>rd</sup> premolar mesial root (a: apical hard tissue regeneration/deposition; b: scar tissue).

**Figure 14** Control NP group, Case 9, Period 92 days, Histopathologically excellent, mandibular right 2<sup>nd</sup> premolar mesial root (a: alveolar bone regeneration/deposition; b: alveolar bone resorption; c: root canal filling material).

**Figure 15** Control NP group, Case 3, Period 83 days, Histopathologically good, mandibular right 3<sup>rd</sup> premolar distal root (a: inflammatory cell infiltration; b: penetrating root canal filling material Neodine).

**Figure 16** Control NP group, Case 25, Period 92 days, Histopathologically poor, mandibular left 1<sup>st</sup> retromolar mesial root.

condition. In addition, newly formed cementum was observed at the root tip, having a good progress with a tendency to close the hole, and so a score of 4 points was given.

Figure 15 shows the histological view of the distal root of the right mandibular 1<sup>st</sup> premolar 83 days post-operatively. The hole at the root tip was still present with slight inflammatory cell infiltration. Hard tissue resorption seems to have stopped but regeneration could not be observed. A score of 3 points was given.

Figure 16 shows the histological view of the mesial root of the left mandibular 1<sup>st</sup> molar 92

days post-operatively. There was alveolar bone resorption without alveolar bone regeneration and lots of inflammatory cells. Remaining inflammation occupied a relatively small area with probable ability for repair. A score of 2 points was given.

Figure 17 shows the histological view of the mesial root of right mandibular 1<sup>st</sup> molar 83 days post-operatively. Significant damages could be observed and destruction still remained with poor results. A score of 1 point was given.

Figure 18 shows the histological view of distal root of the left mandibular 3<sup>rd</sup> premolar 92 days post-operatively. There was granulation tissue and fibrosis at the peripheral slight hard tissue resorption that reached the dentin, creating a significant damage. A score of 0 points was given.

### **Congestion and hemorrhage**

The different pathological findings are summarized in Table 5. The following are additional pathological findings. Congestion was observed in 23/51 cases in VKP (45.1%), 23/42 cases in VNP (54.8%), 9/26 cases in NP group (34.6%). Representative samples showed different degrees classified as minor  $\pm$  (Figure 7A), mild + (Figure 5B) and moderate ++ (Figure 19B). Hemorrhage was observed in 2/51 cases in VKP group (3.9%), 3/42 cases in VNP group (7.1%) and none in NP group. Typical examples eliciting the degree were classified as minor  $\pm$  (Figure 3B) or mild + (Figure 19A). As described above, congestion was frequent in each group and the rate was moderate. NP had the lowest incidence of congestion. This type of change was likely to be accompanied by residual inflammatory changes often thought to entail more reasonable changes during repair. The incidence of hemorrhage was significantly low in each group and there was none in NP group.

### **Inflammatory cell infiltration and suppuration**

Inflammation was observed in 37/51 cases in VKP (72.5%), 34/42 cases in VNP (81%), 25/26 cases in VNP group (81%) and 25/26 cases in NP group (96.2%). Those were classified as minor  $\pm$  (Figure 8A), mild + (Figure 15A), moderate ++ (Figure 9A) and severe +++ (Figure 6C). Suppuration was observed in 11/51 cases in VKP (21.6%), 9/42 cases in VNP (21.4%) and 5/26 in NP group (19.2%). The cases were classified as minor  $\pm$  (Figure 3C), mild + (Figure 20A), moderate ++ (Figure 5A) and severe +++ (Figure 21A). Inflammatory cell infiltration was high in all groups, but a slightly higher incidence was observed in NP group. The extent of inflammation was more or less moderate in all groups. Meanwhile, the incidence of suppuration showed the same rate in each group and was very much lower than the rate of inflammatory cell infiltration, but the degree was moderate to severe. Such a trend was similar to the findings of both Teramoto [3] and Shibuya [4], who performed root canal treatment on infected root canals. When inflammatory changes are compared, suppression of inflammation can be more appreciated than using non-infected canals, which is why this procedure was adopted. Also, suppression of inflammation can be appreciated towards the end of the healing process after a considerable time.

### **Scarring of the periodontal root tip**

Encapsulation of the lesion with granulation tissue, fibrosis and scarring is a kind of tissue remodeling due to damage. This occurs at the periodontal ligament to save the root tip. Scarring was observed in 49/51 cases in VKP group (96.1%), 41/42 cases in VNP group (97.6%) and 26/26 cases in NP group (100%). Those were classified into minor  $\pm$  (Figure 11B), mild + (Figure 4B), moderate ++ (Figure 8B) and severe +++ (Figure 13B). Periodontal tissue scarring at



**Table 4** Statement of Varied Lesions Appearing in Apical Periodontal Tissues

Lesions	Hypermia	Hemorrhage	Infiltration	Suppuration	Cicatrix	Apical Absorption	Apical Regeneration	Alveolar Absorption	Alveolar Regeneration	Root Canal Polyp
Experimental VKP-Group (Number)	51									
Expressed Number (%)	23 (45.1%)	2 (3.9%)	37 (72.5%)	11 (21.6%)	49 (96.1%)	50 (98.0%)	45 (88.2%)	51 (100%)	48 (94.1%)	1 (2.0%)
Level + -	7	1	8	5	0	2	6	0	0	0
+	14	1	11	1	15	23	21	4	12	1
++	2	0	16	4	19	9	15	37	31	0
+++	0	0	2		15	16	3	10	5	0
Experimental VNP-Group (Number)	42									
Expressed Number (%)	23 (54.8%)	3 (7.1%)	34 (81.0%)	9 (21.4%)	41 (97.6%)	42 (100%)	39 (92.9%)	41 (97.6%)	41 (97.6%)	2 (4.8%)
Level + -	9	2	8	5	4	1	10	0	1	0
+	12	1	18	1	9	18	15	6	22	2
++	2	0	6	3	15	12	14	20	15	0
+++	0	0	2	0	13	11	0	15	3	0
Control NP-Group (Number)	26									
Expressed Number (%)	9 (34.6%)	0 (0.0%)	25 (96.2%)	5 (19.2%)	26 (100%)	26 (100%)	12 (46.2%)	26 (100%)	26 (100%)	1 (3.8%)
Level + -	1	0	7	2	0	1	6	0	4	0
+	8	0	11	0	7	9	5	5	12	1
++	0	0	5	2	11	8	1	12	10	0
+++	0	0	2	1	8	8	0	9	0	0

the root tip is an important finding to determine the modifications; however, in root canal treatment, reports revealed that these were minor findings [3, 4]. In this experiment, moderate to severe scarring in each group was seen in the majority of cases, which clearly indicated that the aim of root canal treatment is towards periodontal repair. The difference between the three groups was unacceptable, frequently showing moderate or severe scarring. However, a faster healing rate seemed to be observed in the 2 groups which utilized the ‘simultaneously folded’ technique.

**Hard tissue regeneration at the edge of the root tip**

Regeneration at the edge of dental hard tissue destruction associated with infected root canals was common, as observed by Bhaskar and Rappaport (1971), and by Weine (1976) [23]. This kind of change is shown in Table 5. Hard tissue regeneration was observed in 50/51 cases in VKP group (98%), 42/42 cases in VNP group (100%) and 26/26 cases in NP group (100%). Those were classified into minor ± (Figure 6A), mild + (Figure 3A), moderate ++ (Figure 4A) and severe +++ (Figure 12A). Soft tissue extent and regeneration were similar in all three groups. Meanwhile hard tissue regeneration is an important kind of healing, as reported by Teramoto [3] and Shibuya [4]. Hard tissue regeneration by once resorbed root tip is possible if the infected root canal has been subjected to treatment and sealed with thin lining cement and amalgam restoration. In this experiment, hard tissue regeneration with considerable frequency

**Table 5** Apical Cementum Formation

	Matrix Only	Irregular Formation	Complete Formation	Total Number
Experimental VKP-Group 51 (100%)	4 (7.8%)	9 (17.6%)	9 (17.6%)	22 (43.1%)
Experimental VNP-Group 42 (100%)	6 (14.2%)	9 (21.4%)	5 (11.9%)	20 (47.6%)
Control NP-Group 26 (100%)	0 (0.0%)	8 (30.8%)	1 (3.8%)	9 (34.6%)

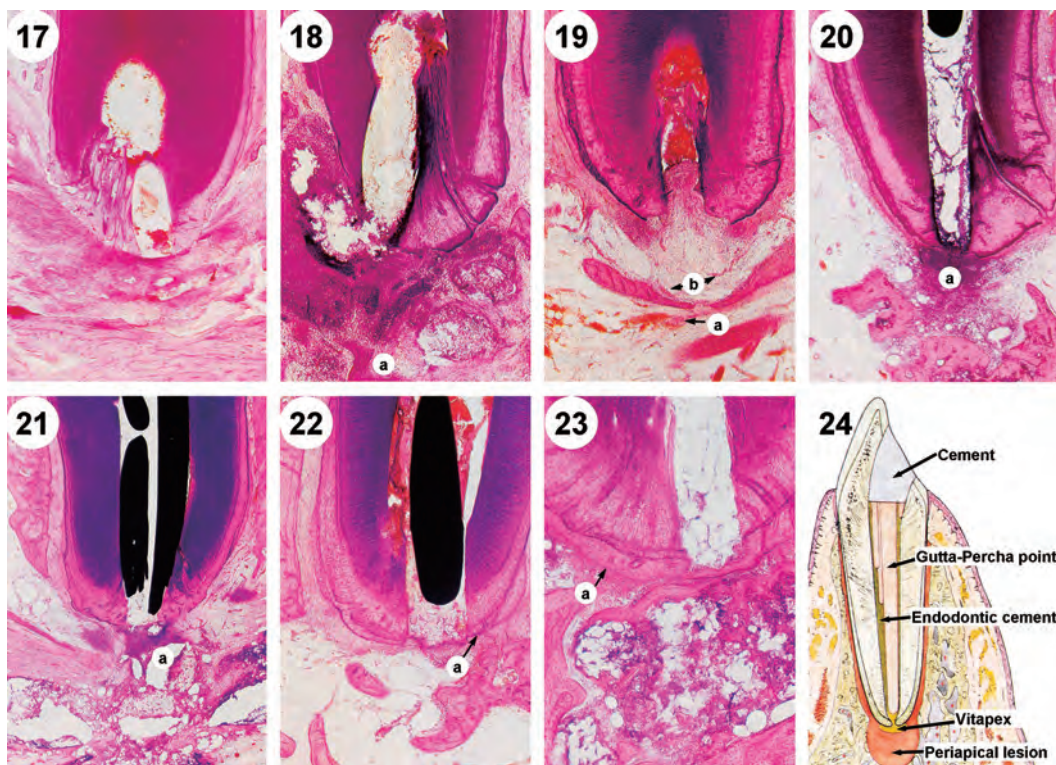
at the root tip was observed in each group. When VKP and VNP were compared to the NP group, a greater difference in the incidence of hard tissue regeneration was observed with the 'simultaneously folded' than with the combined root canal filling method. The addition of hard tissue is shown in Table 5, 45/51 cases in VKP group (88.2%), 39/42 cases in VNP group (92.9%) and 12/26 cases in NP group (46.2%). Those were classified into minor  $\pm$  (Figure 22A), mild + (Figure 9B), moderate ++ (Figure 13A) and severe +++ (Figure 23A). Thus the addition of hard tissue was moderate or severe when the 'simultaneous folded' technique was used instead of the combined technique. Hard tissue regeneration at the root tip had a high rate of healing after root canal treatment, as reported by Teramoto [3] and Shibuya [4]. Among the three root canal filling methods, excellent healing was expected with the 'simultaneously folded' technique.

### Closure of the root tip

Healing after root canal treatment is desired when hard tissue formation penetrates the root tip [3, 4, 24]. Table 5 shows the results. Hard tissue penetration of the root tip was observed in 22/51 cases in VKP group (43.1%), 20/42 cases in VNP group (47.6%) and 9/26 cases in NP group (34.6%). Closure of the root tip could be classified according to the relation of the cementum to the root tip.

1. Only cementum matrix (Figure 9C)
2. Cementum formation and penetration partially closing the root tip (not fully formed) (Figure 8C)
3. Cementum formation completely closing the root tip (fully formed) (Figure 7C)

Seltzer (1971) [24] mentioned that cementum-like structure infrequently closes the root tip completely and that complete closure should have been chosen as the first criteria for the histopathological repair. According to the study of Shibuya (1980) [4], hard tissue closure of the hole at the root tip was achieved by application of Vitapex. Few cases reported that the closure of the hole was due to the union of the addition of new cementum and alveolar regeneration known as ankylosis but such case was not observed in this experiment. According to George et al. (1979) [25], use of calcium hydroxide resulted in complete loss of early periodontal disease at the root tip, although cementum formation at the root tip was hastened by the presence of remaining inflammation, which was also supported by this experiment. Kawakami (1979) [26, 27] reported a high rate of closure of the root apex treated with Vitapex, which was consistent with our results as shown in VKP and VNP groups. The closure rate in Shibuya's experiment [4] was almost the same, with only a slight difference. Teramoto [3] showed a much lower rate of closure in his experimental group using combined root canal filling, compared to our NP group, due to inflammatory changes caused by zinc-oxide eugenol endodontic cement remaining at the



- Figure 17** Control NP group, Case 4, Period 83 days, Histopathologically poor, mandibular right 1<sup>st</sup> retromolar mesial root.
- Figure 18** Control NP group, Case 19, Period 92 days, Histopathologically poor, mandibular left 3<sup>rd</sup> premolar distal root (a: alveolar bone resorption).
- Figure 19** Experimental VNP group, Case 24, Period 83 days, Histopathologically excellent, mandibular left 4<sup>th</sup> premolar distal root (a: hemorrhage; b: hyperemia).
- Figure 20** Experimental VNP group, Case 37, Period 84 days, Histopathologically good, mandibular right 4<sup>th</sup> premolar distal root (a: superlative inflammation).
- Figure 21** Experimental VKP group, Case 45, Period 83 days, Histopathologically poor, mandibular right 1<sup>st</sup> retromolar distal root (a: superlative inflammation).
- Figure 22** Control NP group, Case 6, Period 84 days, Histopathologically excellent, mandibular left 3<sup>rd</sup> premolar distal root (a: apical hard tissue regeneration).
- Figure 23** Experimental VKP group, Case 30, Period 84 days, Histopathologically excellent, mandibular right 3<sup>rd</sup> premolar distal root (a: apical hard tissue regeneration/deposition).
- Figure 24** 'Simultaneously folded' method with paste and combined root canal filling methods.

root tip over a long period. This finding was also reported by Muruzabal et al. (1966) [28, 20, 30, 31, 32]. Sufficient attention should be done in order to prevent spillage of zinc-oxide eugenol endodontic cement outside the root tip.

### Alveolar bone resorption

Alveolar bone resorption in this experiment occurred as healing was taking place and showed no traces of either bone resorption or regeneration. As shown in Table 5, 51/51 cases in VKP group (100%), 41/42 cases in VNP group (97.6%) and 26/26 cases in NP group (100%) were noted. The cases were classified as mild + (Figure 14B), moderate ++ (Figure 6B) and severe

+++ (Figure 18A). The incidence of alveolar bone resorption and regeneration in all three groups was almost the same, suggesting that both conditions happened at the same time in infected root canals in each group. This supports the fact that each root canal treatment was performed under the same conditions. However, Hattori (1975) [6] observed alveolar bone resorption one month after application in almost all cases. Alveolar bone regeneration is as important as hard tissue regeneration as a form of healing of hard tissues. In this study the decrease in inflammation at the root tip showed a tendency towards healing. An array of osteoblasts on the bone wall was destroyed by the addition of bone matrix; the addition of bony plates was recognized by trabecular bone regeneration, which led to the reorganization of the periodontal ligament and alveolar bone regeneration near the edge of the hard tissue formed. As shown in Table 5, there were 48/51 cases in VKP group (94.1%), 41/42 cases in VNP group (97.6%) and 26/26 cases in NP group (100%). Those were classified as minor ± (Figure 10A), mild + (Figure 14A), moderate (Figure 1A) and severe +++ (Figure 7B). From the breakdown of the cases in each method, the incidence was almost the same. When groups were compared, other groups were inferior to some extent, but a difference in intensity was not found in any case. 'Simultaneously folded' root canal treatment in the 2 groups also allowed this strong intensity at the same time as a degree of regeneration. This seems to be the promoting effect of Vitapex application in bone regeneration exemplified by the 3 conditions below:

1. Addition or bone regeneration at the edge of the bony defect
2. Addition or bone regeneration in periodontal bone defects further from the edge of the deep roots
3. Combination of both

Numbers 1 and 2 were observed in combined root canal filling method, and number 3 was reported to show various shapes. However, we reported in Chapter 10 that when Vitapex was used as root canal filling, aside from bone regeneration, proliferation of irregular or porous-like material was observed at the site of alveolar bone resorption at the periodontal root tip. Such bone formation and restoration did not resemble the original shape and the increased in alveolar bone produced a different form, which seemed to be similar to a tumor. In this experiment, alveolar bone regeneration in VKP and VNP groups was similar to the findings of Shibuya [4]. Alveolar bone regeneration and the added cementum, which closed the hole at the root tip, was clearly divided and mediated by periodontal soft tissues. Vitapex was the common agent used in the VKP and VNP groups. Moreover, bone regeneration was similar in that there was no fusion of the cementum to the added alveolar bone showing no ankylosis, making the results interesting. Furthermore, in the NP group treated with combined root canal filling, Teramoto [3] reported that restoration of alveolar bone regeneration was done with porous bone.

### Root canal polyp

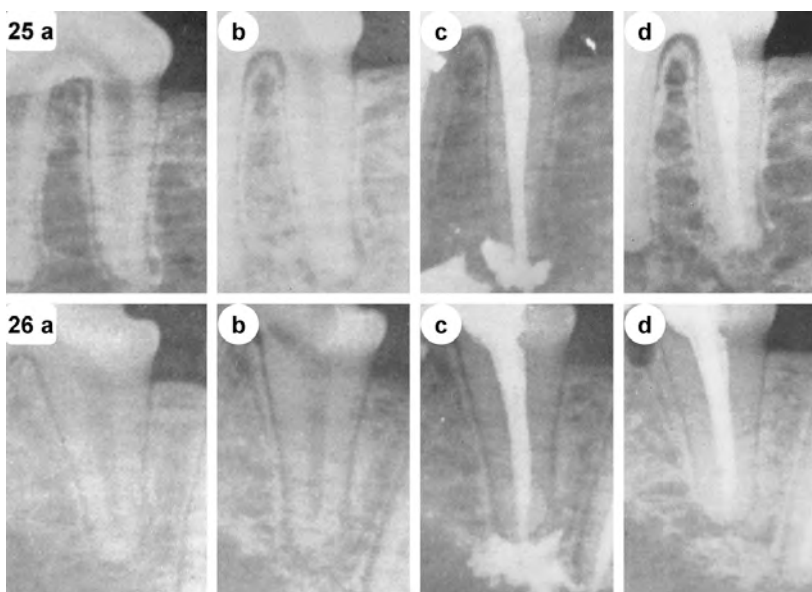
Hyodo (1941) [33] reported the structure of the pathological root canal polyp as 1) granulation tissue, 2) scar tissue and 3) bone-like tissues on the walls of root canals, where in 2 and 3 suggested healing processes. In this experiment, a root canal polyp was observed in 1/51 case in VK group (2.0%), 2/42 cases in VNP group (4.8%) and 1/26 case in NP group (3.8%). Root canal polyps were observed when there was a distance between the root canal filling and the hole, so the relative position of the root canal filling was relevant to the hole. In this experiment, the period of healing of the root canal was not necessarily remarkable by the presence of a root canal polyp and the wound healing process, given that the extent of experimental days was already known from Nanba et al. [34].

### Extent of root canal filling

In the case where a root canal is to be filled with a solid or a combination of paste and solid, overfilling with endodontic sealer or gutta percha should be prevented, and this can be easily identified in radiographs. However, in this experiment it was difficult to know the location of the solid filling materials because of the strong contrast of Vitapex; therefore, the true position of the gutta-percha point could not be determined until Vitapex resorbed after a period of time. The distance between the hole and the gutta-percha points obtained in this way was an interesting result when it came to healing. Underfilling meant that the root canal filling did not reach the lamina dura. When the tip of the root canal filling was located at the boundary corresponding to the lamina or 0.5mm below the root end, in which case did not match the histological sections, it was considered flush. Overfilling meant that the root canal filling material was outside the root tip. As a result, the best method was underfilling, next was flush, and the least effective was overfilling. However, in cases of overfilling and flush, due to overfilling with a substantial amount of Vitapex at the root tip, the 3-month experimental period was considered to be relatively short for a large number of cases with remaining inflammatory response. Root canal filling material which overextended would not significantly affect the final healing after a long period of time. However, it is safe not to overextend in short-term cases.

### The relationship between histopathological findings and x-ray images of root canal filling

According to the study of Hattori (1975) [6], the radiographic and histological relationships



**Figure 25** Experimental VKP group, Case 3, Period 83 days, Histopathologically excellent, mandibular left 1<sup>st</sup> molar (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

**Figure 26** Experimental VKP group, Case 41, Period 83 days, Histopathologically excellent, mandibular right 3<sup>rd</sup> premolar distal root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).



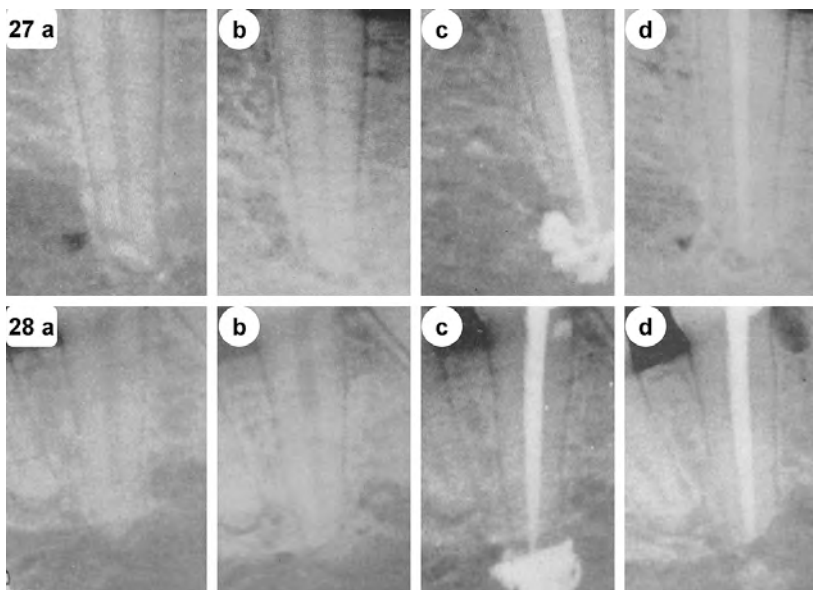
between root canal filling can be divided into 3 forms by their border zones: 1) solid line, 2) non-linear solid boundary or 3) monolithic boundary. In addition, X-ray images of the lamina dura and pathological widening of the periodontal ligament corresponded in a majority cases. In the present study we wanted to describe in detail the results of radiographs taken. First, typical cases for each group were presented and a comparative study on the histopathological findings was undertaken.

### **The relationship between radiographic and histological findings in the VKP group**

Figure 25 is a radiograph of a representative example of a case with a pathological score of 5 points (Figure 1). Lamina dura at the edge of the root before surgery was clearly recognized, but one month after access opening and pulp extirpation, the lamina dura disappeared. Radiolucency was observed 3 months after root canal filling, and the radiolucent part at the root end became as almost opaque as the image of the lamina dura, which tends to heal actively. In addition, the extravasated Vitapex at the edge of the root tip disappeared, suggesting its resorption.

Figure 26 is a radiograph of a representative example of a case with a pathological score of 4 points (Figure 2). One month after access opening and pulp extirpation, there was expansion of the periodontal ligament space and loss of lamina dura exhibiting an amorphous solid white line. Three months later, extravasated Vitapex disappeared and was completely resorbed. There was also a reduction in the periodontal ligament space. Histologically, it was confirmed that the root canal was filled with Vitapex and gutta-percha points.

Figure 27 is a radiograph of a representative example of a case with a pathological score of 3



**Figure 27** Experimental VKP group, Case 34, Period 84 days, Histopathologically good, mandibular right 3<sup>rd</sup> premolar mesial root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

**Figure 28** Experimental VKP group, Case 40, Period 83 days, Histopathologically good, mandibular right 3<sup>rd</sup> premolar mesial root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

points (Figure 3). One month after access opening and pulp extirpation, the root tip was radiolucent with widening of the periodontal ligament space and formation of a lesion at the root tip. Three months later radiographs revealed the appearance of lamina dura with a narrowing of the periodontal ligament space.

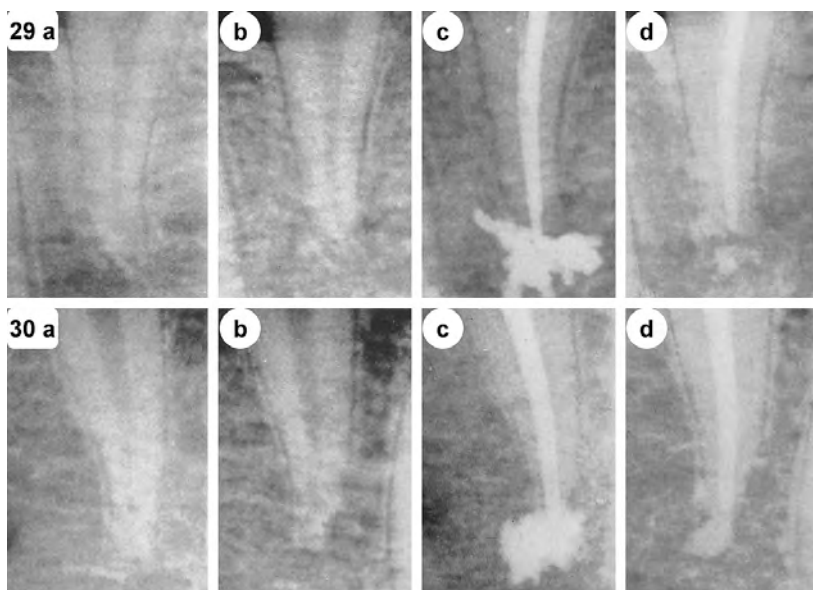
Figure 28 is a radiograph of a representative example of a case with a pathological score of 2 points (Figure 4). Radiographs taken 3 months later showed a slight resorption of the extravasated Vitapex. The pathological score was good, but it did not really coincide with the radiographic findings, and the histopathological relation, could not be discerned.

Figure 29 is a radiograph of a representative example of a case with a pathological score of 1 point (Figure 5). The radiograph taken 1 month after access opening and 3 months after operation caused radiographic impermeability, suggesting a reduction in radiolucency. However, radiographic healing did not correlate with histological findings.

Figure 30 is a radiograph of a representative example of a case with a pathological score of 0 points (Figure 6). Radiographs taken 3 months after root canal filling revealed a reduction in the remaining Vitapex. A partial response to remaining traces of Vitapex was evident, but radiographic findings did not correlate with histological findings. Poor histological condition could not be seen from the radiograph.

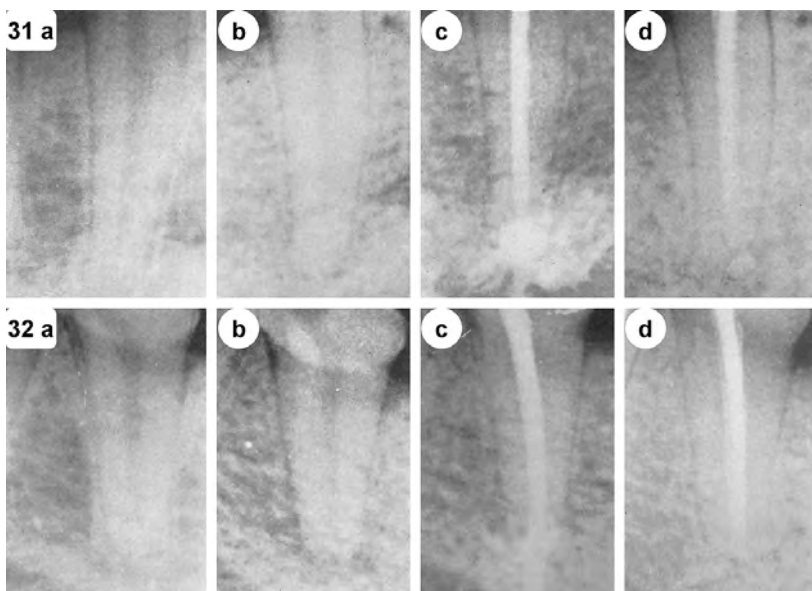
### The relationship between radiographic and histological findings in the VNP group

Figure 31 is a radiograph of a representative example of a case with a pathological score of 5 points (Figure 7). Three months after root canal filling, healing tendency was observed where



**Figure 29** Experimental VKP group, Case 35, Period 84 days, Histopathologically poor, mandibular right 3<sup>rd</sup> premolar distal root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

**Figure 30** Experimental group, Case 49, Period 92 days, Histopathologically poor, mandibular right 3<sup>rd</sup> premolar distal root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).



**Figure 31** Experimental VNP group, Case 29, Period 92 days, Histopathologically excellent, mandibular left 3<sup>rd</sup> premolar mesial root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

**Figure 32** Experimental VNP group, Case 28, Period 92 days, Histopathologically excellent, mandibular left 2<sup>nd</sup> premolar distal root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

the radiographic findings were consistent with histological findings.

Figure 32 is a radiograph of a representative example of a case with a pathological score of 4 points (Figure 8). Three months after root canal filling, radiographic findings were consistent with histological findings where the results were good.

Figure 33 is a radiograph of a representative example of a case with a pathological score of 3 points (Figure 9). Occurrence of lamina dura could be seen 3 months after root canal filling, which agreed with the histological findings.

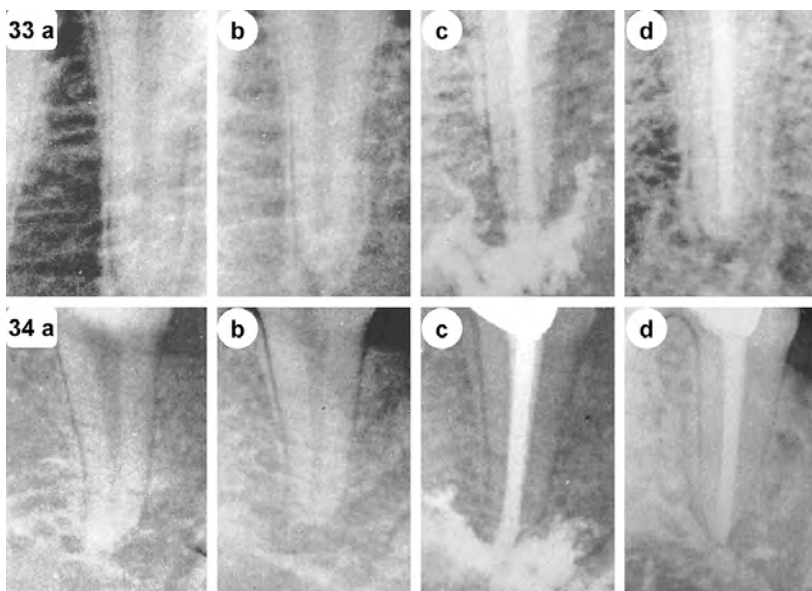
Figure 34 is a radiograph of a representative example of a case with a pathological score of 2 points (Figure 10). Three months after root canal filling, histological findings showed traces of inflammation, which was almost quite similar with the radiograph.

Figure 35 is a radiograph of a representative example of a case with a pathological score of 1 point (Figure 11). Comparing the radiographic and histological findings three months after root canal filling, the hole in the root was observed as well as the presence of root canal polyp. In addition, radiographs showed resorption of the root tip healing but did not match some histological findings.

Figure 36 is a radiograph of a representative example of a case with a pathological score of 0 points (Figure 12). Three months after root canal filling, radiographs showed distinct resorption of the root tip consistent with the histological findings.

### **The relationship between radiographic and histological findings in the NP group**

Figure 37 is a radiograph of a representative example of a case with a pathological score of 5



**Figure 33** Experimental VNP group, Case 32, Period 92 days, Histopathologically good, mandibular left 4<sup>th</sup> premolar distal root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

**Figure 34** Experimental VNP group, Case 38, Period 92 days, Histopathologically good, mandibular right 3<sup>rd</sup> premolar distal root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

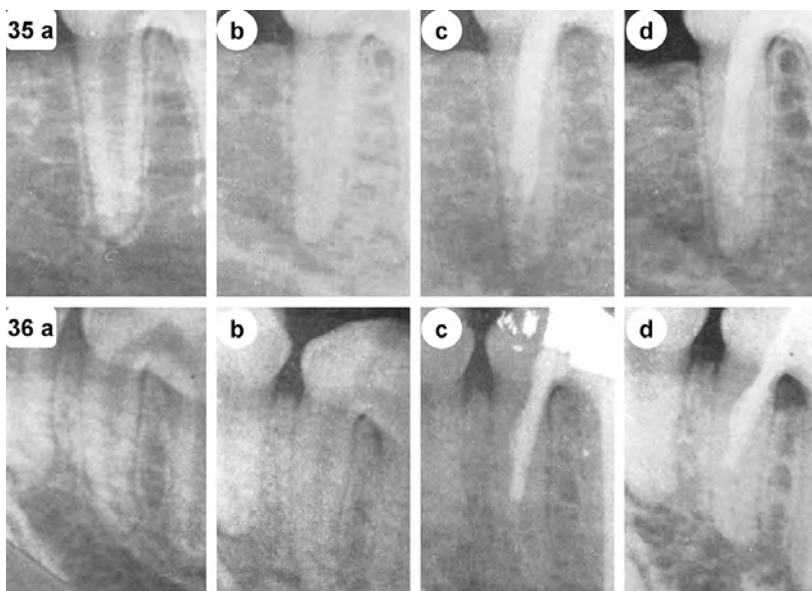
points (Figure 13). Radiographic and histological comparisons three months after root canal filling revealed remaining inflammation at the periodontal root tip not inconsistent with radiographic findings.

Figure 38 is a radiograph of a representative example of a case with a pathological score of 4 points (Figure 14). Three months after root canal filling, radiographs showed no changes in the position of the root canal filling material. However, reduction in the periodontal ligament space, slight radiolucency, fairly radiopaque image and clear active healing were evident. Histological findings confirmed the radiographic findings which showing healing.

Figure 39 is a radiograph of a representative example of a case with a pathological score of 3 points (Figure 15). Radiographs taken three months after root canal filling showed slight reduction in radiopacity of the overfilled root canal filling material. The excess Neodyne outside the root canal tended to disappear reducing the periodontal ligament space. However, resorption at the root tip was noted; histological findings could not confirm radiographic findings, and we acknowledge that they did not match.

Figure 40 is a radiograph of a representative example of a case with a pathological score of 2 points (Figure 16). Three months after root canal filling, radiograph confirmed the presence of extravasated root canal filling Neodyne. The increased and decreased radiopacity at the root tip and the narrowing of the periodontal ligament space were different from the histological findings.

Figure 41 is a radiograph of a representative example of a case with a pathological score of 1 point (Figure 17). Three months after root canal filling, extravasated Neodyne still remained.



**Figure 35** Experimental VNP group, Case 3, Period 83 days, Histopathologically poor, mandibular left 3<sup>rd</sup> premolar mesial root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

**Figure 36** Experimental VNP group, Case 1, Period 83 days, Histopathologically poor, mandibular left 2<sup>nd</sup> premolar mesial root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

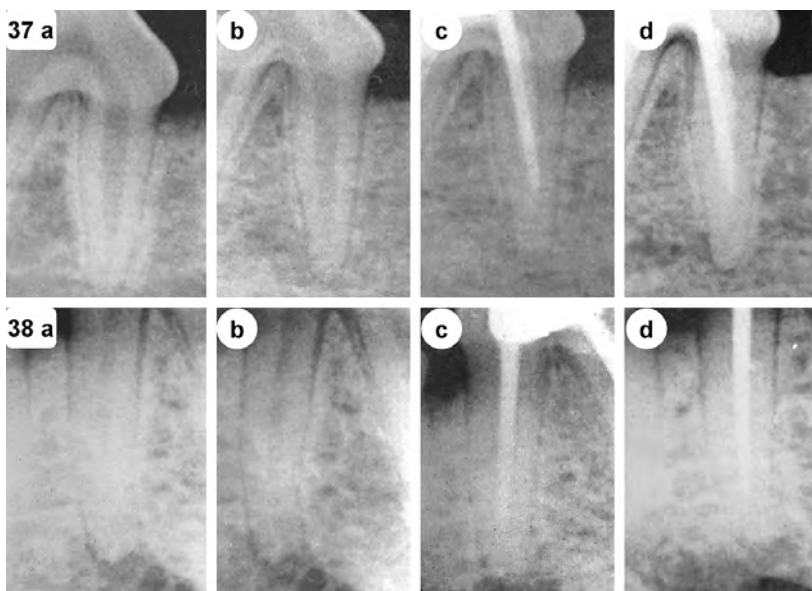
Increased radiopacity of the root tip was observed with a marked healing tendency. On the other hand, a wide range of inflammatory cell infiltration and sepsis at the periodontal root tip could still be observed. This range was consistent with the radiolucent area observed in radiographs and pathological healing over time.

Figure 42 is a radiograph of a representative example of a case with a pathological score of 0 points (Figure 18). Three months after root canal filling, the radiographic findings coincided with the histological findings. Histologically, extravasated Neodyne at the root tip was observed as well as prominent inflammatory cell infiltration and suppuration. Root resorption, which was clearly observed in radiographs, was not clearly observed in histological sections and so a mismatch in the radiographic and histological findings was noted.

### **Relationship between the pathological results and radiographic findings**

When the radiographs taken a month after access opening and three months after root canal filling were compared, most cases were in good condition. The narrowing of the periodontal ligament space and the increasing radiopacity at the root tip indicated healing tendencies. This proves the efficacy and legitimacy of various root canal filling experiments and only few cases obtained a score of less than 2 points. The healing process observed in radiographs can be understood by performing a comparative study of the histopathological findings, which provide a clear picture of the condition.





**Figure 37** Control NP group, Case 2, Period 83 days, Histopathologically excellent, mandibular right 3<sup>rd</sup> premolar mesial root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

**Figure 38** Control NP group, Case 9, Period 92 days, Histopathologically excellent, mandibular right 2<sup>nd</sup> premolar mesial root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

## Discussion and summary of the pathological results

### Pathologic scores

Table 6 shows the summary of the pathological results. Pathological scores for the VKP and VNP groups showed little difference, and both showed good results. Compared to the 2 groups, NP had fair results. The 'simultaneous folded' root canal filling method with Vitapex applied at the apex was done in the VKP and VNP groups but did not seem to be an advantage over the NP group although favorable pathology results were expected. Experiments showed that 'simultaneous folded' root canal filling has potential for future clinical practice.

### 'Simultaneously folded' vs combined root canal filling method

Ishikawa (1977) [18] first developed the 'simultaneously folded' method of root canal filling using Vitapex, and Narita (1976) [36] became an important reference for researchers. A study by Shibuya [4] proved the excellent results in healing at the time of using Vitapex. This experiment compared the conventional combination of root canal treatment with the 'simultaneously folded' method using Vitapex at the root tip. Ingle (1956) [37] reported that the gap between the hole at the root apex and apical terminal could be eliminated by flowable endodontic cement. When used in general root canal treatment, endodontic cement extravasation from the hole often seems to be effective. In this study, extravasation of endodontic cement in the NP group led to poor healing. On the other hand, excellent sealing of the root canal was shown in studies of Marshall and Massler (1961) and other researchers [38, 39, 40]. The advantage of sealing ability of the

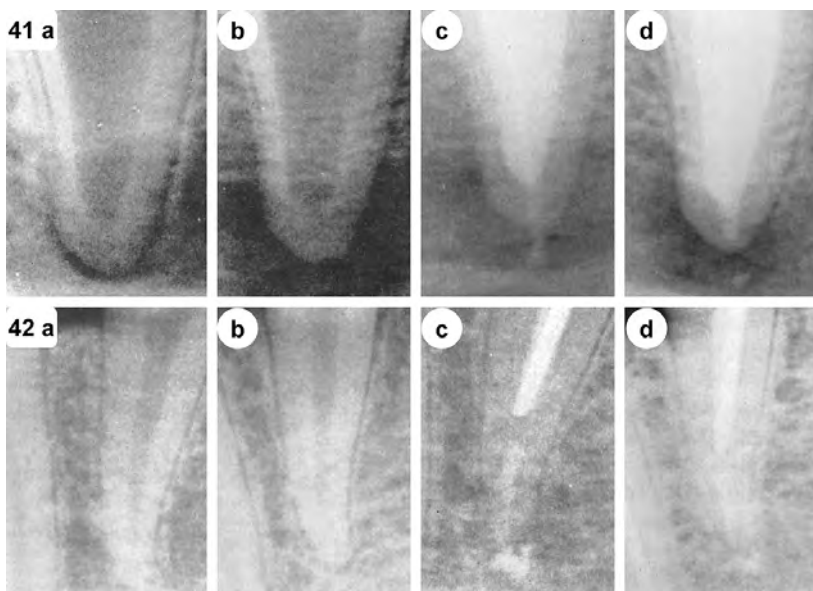


**Figure 39** Control NP group, Case 3, Period 83 days, Histopathologically good, mandibular right 3<sup>rd</sup> premolar distal root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

**Figure 40** Control NP group, Case 25, Period 92 days, Histopathologically good, mandibular left 1<sup>st</sup> premolar mesial root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

**Table 6** Summary of Histopathological Examination

	Number (Avg Point)	Result					
		Excellent		Good		Poor	
		5	4	3	2	1	0
Experimental VKP-Group	51 (3.7)	14	21	9	2	3	2
		35 (68.6%)		11 (21.6%)		5 (9.8%)	
		46 (90.2%)					
		Experimental VNP-Group	42 (3.6)	8	20	8	2
28 (66.7%)				10 (23.8%)		4 (9.5%)	
46 (90.5%)							
Control NP-Group	26 (3.1)			1	10	10	2
		11 (42.3%)		12 (46.2%)		3 (11.5%)	
		23 (88.5%)					



**Figure 41** Control NP group, Case 4, Period 83 days, Histopathologically poor, mandibular right 1<sup>st</sup> retromolar mesial root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

**Figure 42** Control NP group, Case 19, Period 92 days, Histopathologically poor, mandibular left 3<sup>rd</sup> premolar distal root (a: Pretreatment; b: 1 month after the access opening and pulp extirpation; c: Right after filling; d: 3 months after the root canal filling).

root canal and healing ability of calcium hydroxide were the main purpose of the study in which further healing was expected. Results of the experiment seemed to clearly show the efficacy of the 'simultaneously folded' root canal filling method.

## Conclusion

Dogs were used as experimental subjects where periapical lesions were established. Root canals were cleaned followed by obturation. In the VKP group, 51 root canals were filled with Vitapex, KEZ endodontic cement and gutta-percha points subjected to the 'simultaneously folded' root canal filling method. In the VNP group, 42 root canals were filled with Vitapex, Neodyne and gutta-percha points subjected to the 'simultaneously folded' root canal filling method. In the NP group, 26 root canals were filled with Neodyne and gutta-percha points subjected to the combined root canal filling method. The experimental period lasted for 3 months after obturation.

1. Inflammatory changes at the root tip were observed in 72.5% in the VNP group, 81.0% in the VNP group and 96.2% in the NP group, and the cases without inflammatory changes were 27.5% in the VKP group, 19.0% in the VNP group and 3.8% in the NP group. Remaining inflammatory changes decreased in that order, where the NP group had the least. Application of Vitapex slightly helped in reducing periodontal inflammatory changes.
2. There was a high tendency of repair and scarring at the periodontal root tip by the two root canal filling methods. A faster healing rate and higher incidence of moderate to severe scarring was observed in the 'simultaneously folded' root canal filling method.

3. Hard tissue regeneration was found at the root tip in both root canal filling methods, but the amount and frequency was more in the 'simultaneously folded' method.
4. Closure of the hole at the root tip was observed in many cases by newly-formed cementum, but a higher frequency and the extent of closure were observed in the 'simultaneously folded' method.
5. High rates of alveolar bone regeneration occurred in both methods. The 'simultaneously folded' method group had a higher rate but the difference was not significant. Alveolar bone resorption and porous bone-like proliferation or random addition was observed in many cases in the 'simultaneously folded' method, but in the combined method, alveolar plates tended to form on the layer of alveolar bone resorption. Ankylosis is a feature of Vitapex and it has been reported, but in this experiment, ankylosis was not observed and a clear demarcation between the root tip and the surrounding soft tissue was clearly observed.
6. A root canal polyp was observed when there was a distance between the hole and the root canal filling.
7. The experimental period, in general, was short for healing after root canal therapy. Nevertheless, underfilling showed better healing compared to flush or overfilling but eventually seemed to be headed towards healing.
8. Many cases had radiographic findings which correlated with histological findings, but this correlation was not observed in cases having pathologic scores of 2 points, in which case the results were better from the radiographs.
9. The 'simultaneously folded' root canal filling method adopted in this study showed more characteristic morphology of healing than did the conventional method. It promoted closure of the hole at the root tip with relatively rapid alveolar bone regeneration without ankylosis, thereby promoting remodeling and healing of the root end resembling normal healing, which may be clinically effective.

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